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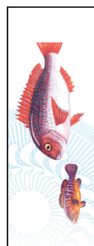
Journal of Fish Diseases 2014
doi:10.1111/jfd.12309



Sea bass *Dicentrarchus labrax* (L.) bacterial infection and confinement stress acts on F-type lectin (DlFBL) serum modulation

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Abstract

The F-lectin, a fucose-binding protein found from invertebrates to ectothermic vertebrates, is the last lectin family to be discovered. Here, we describe effects of two different types of stressors, bacterial infection and confinement stress, on the modulation of European sea bass *Dicentrarchus labrax* (L.) F-lectin (DlFBL), a well-characterized serum opsonin, using a specific antibody. The infection of the *Vibrio alginolyticus* bacterial strain increased the total haemagglutinating activity during the 16-day testing period. The DlFBL value showed an upward regulation on the first, second and last days and underwent a slight downward regulation 4 days post-challenge. In contrast, the effect of confinement and density stress showed a decrease in the plasma concentration of lectin, ranging from 50% to 60% compared with the control. The modulation of DlFBL is in line with the hypothesis that humoral lectins could be involved and recruited in the initial recognition step of the inflammation, which leads to agglutination, and the activation of mechanisms responsible for killing of the pathogens.

Keywords: confinement stress, *Dicentrarchus labrax*, F-type lectin, infection, modulation, teleost.

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Introduction

Lectins are a group of sugar-binding proteins that recognize specific carbohydrate structures through specific domains (CRDs), resulting in the regulation of various cell functions and interactions via glycoconjugates. They mediate several functions including agglutination, immobilization, recognition and opsonization of microbial pathogens, and they complement pathway and phagocyte activation (Vasta, Ahmed & Odom 2004). Lectins are able to recognize cell types and development stages because cells often display altered surface glycoproteins and glycolipids depending on their physiological and pathological conditions.

The F-type lectins (FTLs) constitute the lectin family that has been identified and structurally characterized in teleosts (Bianchet *et al.* 2002, 2010). The FTL family is constituted by a large number of proteins exhibiting greater multiples of the F-type motif in the CRD, arranged either in tandem or in mosaic combinations with others, including a putative transmembrane receptor; an extensive functional diversification of this lectin family from prokaryotes to amphibians is thus suggested (Odom & Vasta 2006).

In teleost fish, well-characterized members of this family are the serum lectins from the Japanese eel, *Anguilla japonica* (Temminck & Schlegel) (Honda *et al.* 2000), sea bass *Dicentrarchus labrax* (Cammarata *et al.* 2001; Salerno *et al.* 2009; Parisi *et al.* 2010), striped bass *Morone saxatilis* (Walbaum, 1792) (Bianchet *et al.* 2002) and sea bream *Sparus aurata* (L.) (Cammarata *et al.* 2007, Cammarata *et al.* 2012).

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Journal of Fish Diseases

One of the major problems encountered during the culture of aquatic organisms is the occurrence of disease. Outbreaks may be related to adverse environmental conditions or occur where the cultured organism comes into contact with a critical mass of a pathogen within the water column. The fish innate immune response is the first barrier against pathogens. It has both cellular and humoral components, which carry out their protective function, for at least the time required at the start of the antibody inducible response, using accessory cells able to present antigens to lymphocytes (Magnadottir 2006).

Lectins are believed to mediate pathogen recognition in fish immune systems with important roles in innate immune response (Vasta *et al.* 2011).

In addition to the function of defence against micro-organisms, there is evidence that the fish lectins play an important role in fertilization, morphogenesis, mitogenic and antiproliferative activities (Dong *et al.* 2004; Russell & Lumsden 2005).

Specific interactions between these lectins and complex carbohydrates (glycoproteins, glycolipids, polysaccharides or proteoglycans) are also involved in numerous basic phenomena, such as embryonic development, intracellular trafficking, cell–cell and cell–matrix recognition, cell homing, endocytosis, phagocytosis, inflammation, and the metastatic spread of cancer cells (Duverger *et al.* 2010). Moreover, sugar-specific interaction induced pro-inflammatory cytokines in rainbow trout and caused alterations of gene expression in Burkitt's lymphoma cells in channel catfish *Ictalurus punctatus* (Rafinesque) (Ogawa *et al.* 2011). Many fish lectins agglutinate marine pathogen bacteria, such as *Vibrio* genus (Kamiya, Muramoto & Goto 1988), and have opsonic and cytotoxic activities (Ogawa *et al.* 2003; Nakamura *et al.* 2006).

Physiological stress and physical injury are the primary contributing factors of fish disease and induce a number of responses involving all three regulatory systems: neural, endocrine and immune. Many potential fish pathogens are continually present in the water, soil, air and fish. To fight infection or survive stress, fish use effectors of the innate immune system. Several external and internal factors can influence the activity of innate immune parameters such as temperature changes, handling and crowding stress (Magnadottir 2006). Humoral parameters include growth inhibitors, lytic enzymes, natural antibodies, cytokines, chemokines, antibacterial peptides and lectins (Uribe *et al.* 2011).

The presence of terminal L-fucose as non-reducing terminal residue on various glycoproteins and glycolipids is a key moiety mediating many cellular interactions (Vasta *et al.* 2012). Expression of fucose-containing antigens has been observed to dramatically increase during inflammation (Becker & Lowe 2003). In mammals, Fuc-TVII, a fucosyltransferase, is essential for proper recruitment of neutrophils and T cells to inflammatory sites, and lymphocyte trafficking to secondary lymphoid organs (Maly *et al.* 1996; Smith *et al.* 1996).

As with other lectins, teleosts F-lectins are present in the plasma of unchallenged individuals and serve as acute phase reactants, rapidly increasing their plasma concentration in response to stress, injury or infection (Vasta *et al.* 2011).

In hepatocytes of the *Anguilla japonica*, FTLs are rapidly upregulated by infectious challenge, thus behaving as true acute phase reactants (Tasumi *et al.* 2002).

Previously, we demonstrated that fish binary CRD F-type lectins play an active role as recognition factors in innate immune functions by enhancing bacteria phagocytosis of the sea bass DlFBL leucocytes (Salerno *et al.* 2009) and SAuFBL32 (Cammarata *et al.* 2011).

The current paper attempts to evaluate the effect of two different types of stressors, bacterial infection and confinement stress in aquaculture, on the modulation of the DlFBL, a specific *D. labrax* fucose-binding lectin.

Materials and methods

Bacterial suspensions

Vibrio alginolyticus strain cultured in tryptic soy broth containing 3% (w/v) NaCl at 25 °C, 120 rpm, was harvested at the stage of exponential growth. The density of bacteria was estimated from serial dilutions plated on tryptic soy agar 3% (w/v) NaCl and counting colony-forming units. To kill bacteria, formaldehyde was added to the stock suspension to give 2% final concentration and shaken overnight at 21 °C, 120 rpm. After centrifugation (6000 ***g***, 15 min, 4 °C), the killed bacteria were washed three times with sterile phosphate-buffered saline (PBS), pH 7.2, suspended 1×10^9 cells mL^{-1} in PBS containing 0.1% (w/v) gelatin and kept at 4 °C until use.

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Inflammation and plasma sampling

Specimens of *D. labrax* (n.52) were maintained in Ittica del Mediterraneo (Trapani, Italy) with an average body weight of 250 g and in the same farm were separated into three groups and placed in separate tanks with temperature controlled at 18 °C, 8 ppm salinity and 37 ppm oxygen in sea water.

Before the challenge with *V. alginolyticus* suspension, fishes were anesthetized with 0.05% 3-aminobenzoic acid ethyl ester (MS-222 Sigma) in seawater. Inflammation was carried out on 24 specimens of the first group by intraperitoneal injection of 250 μL of Hanks balanced salt solution (190 mM NaCl, 5 mM KCl, 5.5 mM Glucose, 0.5 mM KH_2PO_4, pH 7.6) containing killed *V. alginolyticus* (2.5×10^6).

To perform the positive control, 24 of the sea bass from the second group were injected with 250-μL HBSS buffer. The third group of four specimens was not injected and was used as a negative control.

Dicentrarchus labrax blood was then collected by caudal venous puncture using 2-mL syringes containing 100 μL of heparin as anticoagulant 0, 1, 2, 4, 8 and 16 days post-challenge. Four animals per time were subjected to blood sampling. Plasma was separated by centrifugation (10 min, 800 ***g***, 4 °C), divided in aliquots and stored at −20 °C until use for assays.

The experiments were performed in full compliance with the national guidelines (D.Lgs 116/92 and subsequent amendments) and the international European Commission Recommendation guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC).

Confinement and density stress condition

A total of 90 sea bass with an average body weight of 250 g were obtained from Ittica Trappeto distributed in 3 tanks.

Tanks were maintained in department aquaria at 18 °C (8 ppm salinity and 37 ppm oxygen in sea water) for 10 days of acclimation. Each tank was assigned a stocking density. The first had a stocking density of 10 kg m^{-3} (low density), and the second tank had a stocking density of 60 kg m^{-3} (high density). In the control tank, a density of 30 kg m^{-3} of free-swimming fish was maintained. At each stocking density, fish were manually fed with artificial feed Trouvit of the Hendrix SpA. The experiment lasted for 48 h, after which the fish were anesthetized with 0.05% MS-222 (Sigma) in seawater. Similarly to the inflammation experiment, all blood samples were collected by caudal puncture using 2-mL syringes containing 100 μL of heparin as anticoagulant.

Erythrocytes and haemagglutination assay

Rabbit erythrocytes (RE) in Alsever solution (0.42% NaCl, 0.8% Na-citrate dihydrate, 0.045% citric acid monohydrate, 2.05% D-glucose, pH 7.2) were provided by the Sicilian Zooprophylaxis Institute (Palermo, Italy).

Haemagglutinating activity (HA) was determined using microtitre plates in which 25 μL plasma serially diluted in 0.1% gelatin in PBS (KH_2PO_4 6 mM, Na_2HPO_4 0.11 mM, NaCl 30 mM, pH 7.4) was mixed with the same volume of erythrocyte suspension. HA was examined after incubation at 37 °C for 1 h, and the titre was expressed in terms $\log_2$ of the reciprocal of the highest dilution showing agglutinated erythrocytes. For haemagglutination assays and chemical–physical characterizations, 1/25 diluted plasma was used.

Protein content estimation

Quantitative protein analysis was performed according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

Polyacrylamide gel electrophoresis

One-dimensional SDS-PAGE was applied to resolve proteins of different molecular size according to the method of Laemmli (1970) using a 4% (stacking) and a 10% (separating) polyacrylamide gel. Total plasma proteins were electrophoresed for 50 min at 190 V using a Bio-Rad mini gel kit.

Preparation of anti-DlFBL antisera

Specific antibodies were prepared and assayed according to Cammarata *et al.* (2001). Bands identified in the SDS-PAGE gels as the purified DlFBL were excised and pooled, suspended in distilled water and passed repeatedly through a syringe. Anti-DlFBL antibodies were grown in rabbits by Medprobe (Norway), with a coarse

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suspension of the gel pool containing DlFBL (50 μg) as the antigen. To control for antibody specificity, the antiserum was absorbed with the purified lectin. Briefly, 500 μL of anti-DlFBL antiserum was mixed with 100 μL of the affinity chromatography DlFBL (50 μg protein content), incubated overnight at 4 °C and centrifuged at 27 000 ***g*** for 1 h at 4 °C. Specificity of the anti-DlFBL antibodies was validated by comparing the activity of the diluted (5:1 in PBS) antiserum with the adsorbed one.

Immunoblotting analysis

Proteins separated by SDS-PAGE (10%) were electroblotted onto a nitrocellulose membrane. The gels were prepared in blotting buffer (20 mM Tris–HCl, 192 mM glycine, 20% methanol, pH 8.8), and a semi-dry blotting bath (Bio-Rad Laboratories) was used (0.8 mA cm^{-2} for 75 min). The filter membrane was soaked in blocking buffer (PBS containing 3% BSA and 1% Tween-20), incubated with anti-DlFBL antibody (1:400 in washing buffer, 0.1% BSA) for 1 h, washed with blocking buffer and incubated with sheep anti-rabbit IgG-alkaline phosphatase conjugate (1:15 000 in washing buffer, 0.1% BSA) for 1 h. Finally, the nitrocellulose membranes were washed with PBS-Tween-20 (3 × 15 min) and developed with the BCIP/NBT liquid substrate system.

Dot blot analysis

Dot blot methodology differs from traditional Western blot techniques in that it does not separate protein samples using electrophoresis. Sample proteins were spotted onto membranes and hybridized with antibody using Bio-Dot microfiltration manifold (Bio-Rad). Membrane was incubated in blocking solution for 1 h. After incubation, the membrane was treated with primary antibody solution of antiDlFBL (1:400 diluted in blocking solution, 0.1% BSA), for 2 h at room temperature. After washing, the membrane was incubated with sheep anti-rabbit IgG secondary antibody–alkaline phosphatase enzyme conjugate solution (in blocking solution, 0.1% BSA) for 1 h and washed in PBS-Tween-20 (3 × 10 min) and developed with the BCIP/NBT liquid substrate system until spots were visible. The reaction was stopped by rinsing the membrane in distilled water.

Densitometric analysis

At the end of the immunoblotting, or dot blotting, reactions, the nitrocellulose membranes were washed in distilled water and dried before being used for the image bands detection. The comparison between the bands based on the colorimetric variable intensity was carried out using the software AlphaEaseFC (Alpha Innotech) to obtain the integrated density value (IDV). Data were recorded on matrices and subsequently processed. To standardize the densitometric data, the acquisition of the images and the selection of the bands were conducted maintaining the same criteria.

Results are presented as the arithmetic mean of optical density of the three experiments ±SD. Data were analysed with the Student's *t*-test for statistically significant difference ($P < 0.05$).

Results

Haemagglutinating activity

Fish plasma collected during the experimental trial showed a variable agglutinating activity towards rabbit erythrocytes in a time-dependent manner after bacterial intraperitoneal injection (Fig. 1). In not-injected specimens, the cross-reactivity indicated by Log_2 of agglutinating activity (HA) is clearly lower (5.5) than in samples injected with buffer or bacterial suspension.

The HA of the plasmas subjected to infection is significantly higher than plasma inoculate only with buffer until 10 days post-challenge. Thereafter, the agglutinating activity of plasmas infected appears similar to the control.

Four days post-challenge, the specimens infected with *V. alginolyticus* and treated with buffer displayed lower values of haemagglutinating activity (equal to 6 and 5.67, respectively). Greater values were detected on the second day in specimens infected with bacteria and on the 16th day in the fish control. An additional increase is shown after 8 days post-challenge with a value of 6.75 for the infected samples and 6.50 for control samples.

Anti-DlFBL antibody reaction after bacterial infection

Before using plasma from infected organisms, we verified the cross-reactivity of the polyclonal

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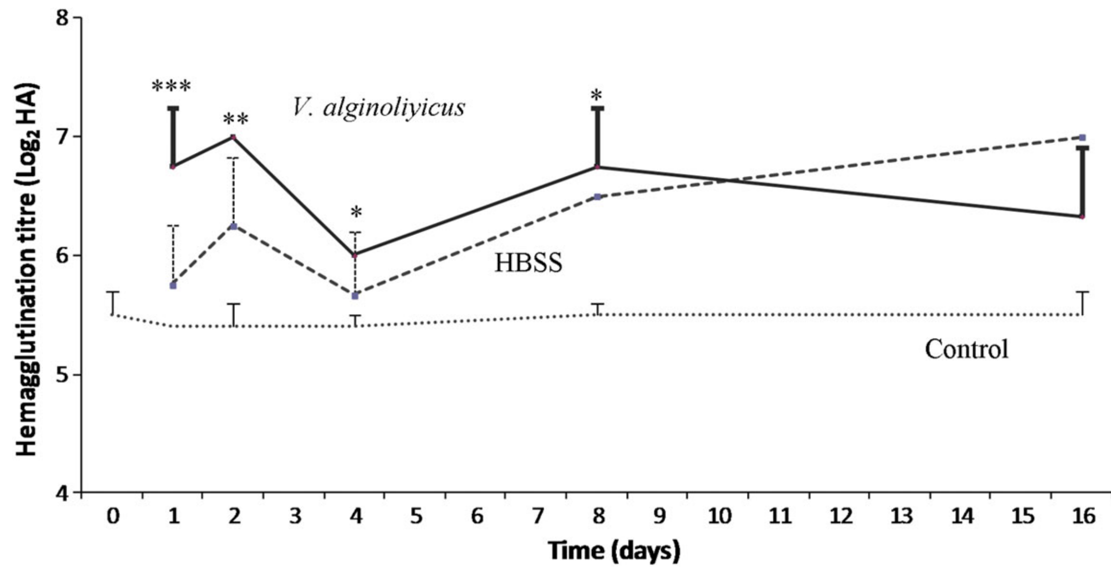

Figure 1 Haemagglutinating activity of *Dicentrarchus labrax* plasma during bacterial challenge. Continuous line shows haemagglutinating activity towards rabbit erythrocytes (RRBC) calculated as logarithm of the agglutination titre (HA) of *D. labrax* plasma samples after intraperitoneal *Vibrio alginolyticus* injection. Plasma was collected 1, 2, 4, 8 and 16 days after bacterial challenge. Dashed line shows activity from organisms injected with HBSS medium (control). Dotted line indicates non-stimulated response. Data are represented as the mean ±SD, and the significant values were calculated by Student's *t*-test: $*P < 0.05$;$**P < 0.01$,$***P < 0.001$.

anti-adult DlFBL antibody in a Western blotting assay. *Dicentrarchus labrax* plasma and DlFBL analysed by SDS-PAGE (10%) showed a single 34-kDa band under reducing conditions that reacted with antibody (Fig. 2a–d).

Thus, all blood samples obtained from injected fish were subjected to electrophoresis on 10% SDS gel and then to Western blotting analysis. After staining of the sheets with the polyclonal antibody anti-DlFBL, a variable immune reaction was detected in samples taken on five different occasions post-challenge.

Densitometric analysis on the Western blot membranes was carried out by Alpha Easer application on three lanes referred to three different sea bass specimens. The calculated integrated value of density (IDV) was used to estimate changes of blood DlFBL levels (Fig. 2.1–2.3).

As shown by the curve in Fig. 3, 24 h post-challenge, the cross-reactivity of plasma infected with bacteria increased to 80% and 33% compared with samples from non-injected fish (negative control) and the positive control, respectively. In particular, DlFBL shows a higher significant value after 1, 2 and 16 days post-stimulation. The DLFBL value in infected blood increases to 8.25% from the first to the second day and then decreases to 21% after the fourth day post-challenge. The DLFBL level continues to increase slowly until the 16th day, the end of the experiment, where the same value as on the first day is recorded. After 1 and 2 days post-challenge, the curve relating to the positive control shows values of antibody cross-reactivity of <25% and 20% compared with infected plasmas. Instead, values increase to 20% at 4 and 8 days post-injection. At the end, the cross-reactivity decreases to 86% compared with plasmas infected.

Effect of density and confinement stress on lectin modulation

Using the experimental design of density and confinement stress, densitometric analysis of dot blots allowed us to quantify the cross-reaction between plasma and the specific antiDlFBL antibody. The average values quantified in five different *D. labrax* specimens are higher in the control than in samples from organisms subjected to stress. In the confinement condition, the level of lectin in blood decreases to 42% with respect to the control value (Fig. 4b), while in the condition of density stress, the amount of lectin is equal to 50% (Fig. 4c). These specificity of this results have

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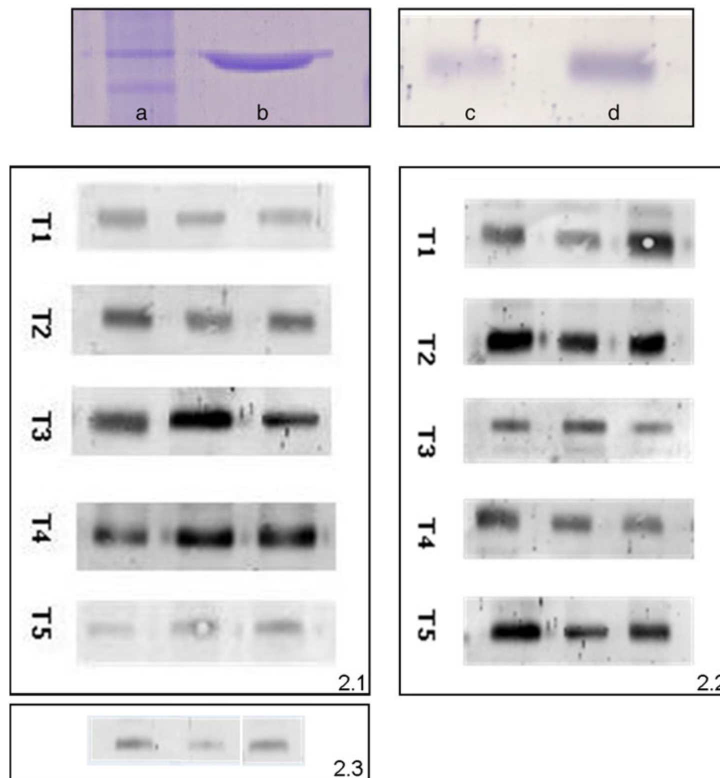

Figure 2 Immunoblotting DlFBL detection in untreated and challenged specimens. SDS-PAGE analysis (10% acrylamide) under reducing conditions of *Dicentrarchus labrax* plasma (lane a) and DlFBL fraction purified (lane b) stained with Coomassie blue. Immunoblotting of the whole plasma (lane c) and DlFBL (lane d) carried out with anti-DlFBL antibodies. 2.1. Immunoreaction with anti-DlFBL antibody of three individual sea bass sera on days 1 (T1), 2 (T2), 4 (T3), 8 (T4) and 16 (T5) post-challenge with *V. alginolyticus*. 2.2. Controls of three individual sea bass sera on days 1 (T1), 2 (T2), 4 (T3), 8 (T4) and 16 (T5) post-injection with HBSS solution. 2.3. Control, sample before treatment.

been also confirmed by electrophoresis and immunoblotting (data not showed).

Discussion

Protein–carbohydrate interactions have important roles in two distinct aspects of the immune response, pathogen recognition and cellular interactions that lead to pathogen neutralization (Weis, Taylor & Drickamer 1998). Lectin receptors play an important role in the innate immune response by recognizing and binding specific carbohydrate moieties (usually a non-reducing terminal monosaccharide or oligosaccharide) on the surface of potential pathogens through CRDs (West & Goldring 2004; Duverger *et al.* 2010). CRDs, in combination with other domains, can recognize carbohydrate moieties and induce agglutination, immobilization, complement-mediated opsonization and lysis (Weis *et al.* 1998; Ogawa *et al.* 2011).

The F-type lectin is the most recent lectin family (FTL) to be identified and structurally characterized in teleosts (Odom & Vasta 2006; Bianchet *et al.* 2002, 2010; Salerno *et al.* 2009; Parisi *et al.* 2010). The FTL family comprises a large number of proteins exhibiting greater multiples of the F-type motif, arranged either in tandem or in mosaic combinations with other domains, including a putative transmembrane receptor, that suggests an extensive functional diversification of this lectin family from prokaryotes to amphibians (Bianchet *et al.* 2002; Odom & Vasta 2006).

The structure and arrangement of the N-CRD and C-CRD result in a binding surface with high potential to cross-link fucosylated glycans. This, in turn, supports the results that binary tandem CRD FTLs function as opsonins (Salerno *et al.* 2009) by cross-linking sugar structures displayed by microbial pathogens and glycans on the surface of phagocytic cells from the host (Bianchet *et al.* 2010).

Specific lectin–carbohydrate interaction has been proven for several fish parasites (Xu, Klesius & Shoemaker 2001; Kim, Olson & Dahms 2009; Buchmann 2001; Buchmann & Lindenstr 2002; Tsutsui *et al.* 2003; Woo 2001, 2003). The presence of carbohydrate terminals specifically detected by lectins at the parasite membrane and host–parasite interface of the myxozoans *Tetracapsuloides bryosalmonae* (Canning), *Myxobulus cerebralis* (Hofer) and *Enteromixum scophthalmi* (L.) suggests

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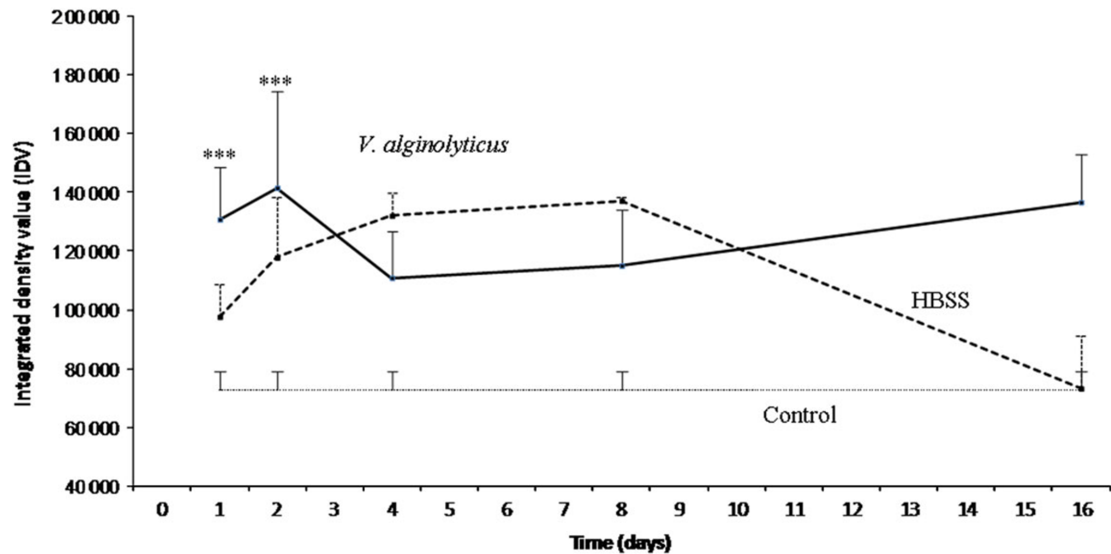

Figure 3 Blood DlFBL integrated density evaluation during induced inflammation with *V. alginolyticus*. Integrated density values (IDVs) calculated for bacterial injected and non-injected samples by Alpha Easer software application. Continuous line indicates DlFBL concentration values in *V. alginolyticus*-challenged specimens. Dashed line shows the DlFBL concentration value in fishes injected with HBSS solution. Dotted line indicates non-stimulated response. The data are represented as the mean of three samples ±SD. The significant values were calculated by Student's *t*-test: $^{***}P < 0.001$.

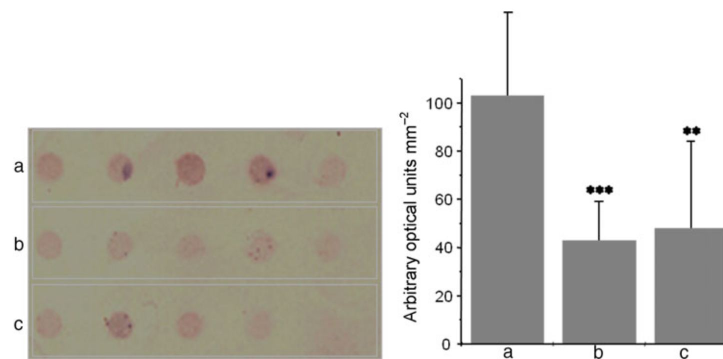

Figure 4 Blood DlFBL evaluation in confinement and high-density conditions. Immunoreaction in dot blot analysis with DlFBL antibody of five different samples not treated (Control) (a), subjected to confinement (b) and density (c) stress conditions. On the right, values of the arbitrary optical units/mm^2 calculated by densitometric analysis are shown. Columns a: control, b: confinement stress, c: high-density condition. The data are represented as the mean of five samples ±SD. The significant values were calculated by Student's *t*-test: $^{**}P < 0.01$,$^{***}P < 0.001$.

a role in host–parasite interactions (Morris & Adams 2004; Knaus & El-Matbouli 2005; Redondo *et al.* 2008). Rhamnose-binding lectins (RBLs) recognize various kinds of pathogens in inflammatory sites throughout the blood circulation and enhance their phagocytosis by binding on the leucocyte surface (Watanabe *et al.* 2009).

Lectins specific for fucose or rhamnose found in *D. labrax* serum, DlFBL and DlRBL play a role in immune recognition of microbial pathogens and facilitate their clearance by phagocytosis (Salerno *et al.* 2009; Cammarata *et al.* 2014). Indeed, the exposure of formalin-killed *E. coli* to DlRBL enhanced their phagocytosis by peritoneal macrophages.

In the current study, we have found that the infection bacteria modulated the general plasma agglutinating activity. Injections of *V. alginolyticus*

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bacterial strains, in fact, stimulate the agglutination titre towards rabbit erythrocytes more than injections of HBSS buffer.

Results of Western blotting and densitometric analysis showed that the expression of DlFBL in plasma undergoes first an increase in the 24 h after the bacteria inoculation and then a decrease on the fourth day. From the fifth day onwards, the concentration of the lectin increases until the 16th day, the end of the experiment. The concentration of DlFBL is higher in the blood of control specimens after the fourth and eighth days of treatment.

In this study, we have found that density and confinement stress acts very differently on the concentration of DlFBL than bacterial stimulus does. In fact, in high-density confinement conditions, the lectin present in the systemic circulation sustains a decrease of about of 60% and 50%, respectively. Contrarily, the bacterial challenge causes an upregulation of DlFBL in the blood of sea bass compared with the control condition and a modulation during the 16 days of experimental trial.

In *Lepeophtheireus salmonis* (Kroyer) experimental infection trials, upregulation of C-type lectin domain family 4 member M (clec4m) occurred in pink salmon *Oncorhynchus gorbuscha*, (Walbaum), whereas upregulation of mannose-binding protein occurred in chum salmon *Oncorhynchus keta* (Walbaum) (Sutherland *et al.* 2014).

Results with LPS injection on roughskin *Trachidermus fasciatus* (Heckel) from Yang *et al.* (2012) reported a strong upregulation of expression of a β-galactoside-binding lectin (TfGal) mRNA in the hemocytes and the skin at 2 h post-challenge, and in the liver at 2–6 h and 96 h post-challenge.

L-Rhamnose-binding lectins (RBLs) in channel catfish also undergo upregulation following a challenge with the bacterial pathogen *Flavobacterium columnare* (Davis). Furthermore, the magnitude of RBL upregulation positively correlated with disease susceptibility (Thongda *et al.* 2014).

Some pentraxins behave as acute phase reactants in teleosts, rapidly increasing their plasma concentration in response to stress, injury or infection, whereas in others, pentraxins are constitutive proteins present at high concentrations in the normal plasma (Lund & Olafsen 1999).

Generally, the expression varied according to both pathogen and tissue type, suggesting that fish lectins may exert disparate functions or exhibit distinct tissue-selective roles in the host immune response to bacterial pathogens or other types of stress.

It has been demonstrated that rearing fish at inappropriate stocking densities may impair the growth and reduce immune competence due to factors such as social interaction and the deterioration of water quality, which can affect both the feed intake and conversion efficiency of the fish (Ellis *et al.* 2002). Vazzana *et al.* (2002) demonstrated that subjecting European sea bass to very high density and confinement in various aquaculture procedures induced a period of depression in the innate immune responses, detected at the cellular level by the depression of the cytotoxic activity of leucocytes.

The opsonic and agglutinating activity of plasma lectins mediates not only agglutination and immobilization of potentially pathogenic micro-organisms, but may also promote their phagocytosis and clearance from circulation, thereby playing a key role in host defence against infectious challenge and sensitivity to stress events.

Our results are in line with the hypothesis that humoral and cell-associated fish lectins can be responsible for the initial recognition step in the first line of internal defence, which leads to agglutination, endocytosis by phagocytic cells and the activation of protease cascade resulting in clotting or complement-mediated killing of the pathogens.

Acknowledgements

This work was supported by grants from MIUR, and MC RITMARE project SP2-WP4-AZ3-UO3 (CNR and CONISMA).

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Received: 1 June 2014
Revision received: 10 August 2014
Accepted: 13 August 2014

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